

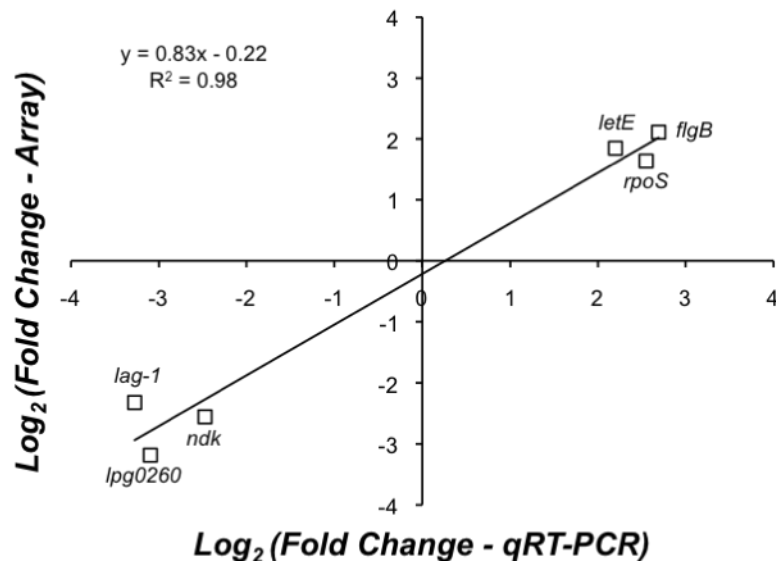
Supplemental Table 1. Select list of transcripts down-regulated 90 min after *relA*^{L-p} induction.

Gene Name / Function	Gene.ID	Annotation	ppGpp ⁰ <i>preA</i> ^{L-p} / <i>pempty</i>
<u>ATP Synthesis</u>			
<i>atpC</i>	<i>lpg2981</i>	Highly similar to H ⁺ -transporting ATP synthase epsilon chain	-2.48
<i>atpD</i>	<i>lpg2983</i>	Highly similar to H ⁺ -transporting ATP synthase beta chain	-2.54
<i>atpG</i>	<i>lpg2984</i>	Highly similar to H ⁺ -transporting ATP synthase chain gamma	-3.00
<i>atpA</i>	<i>lpg2985</i>	Highly similar to H ⁺ -transporting ATP synthase chain alpha	-2.67
<i>atpH</i>	<i>lpg2986</i>	Highly similar to H ⁺ -transporting ATP synthase chain delta	-3.91
<i>atpF</i>	<i>lpg2987</i>	Highly similar to H ⁺ -transporting ATP synthase chain b	-2.99
<i>atpE</i>	<i>lpg2988</i>	Highly similar to H ⁺ -transporting ATP synthase chain c	-2.69
<i>atpB</i>	<i>lpg2989</i>	Highly similar to H ⁺ -transporting ATP synthase chain a	-7.52
<i>atpI</i>	<i>lpg2990</i>	Highly similar to H ⁺ -transporting ATP synthase subunit i	-6.54
<u>Membrane Modification</u>			
<i>fabZ</i>	<i>lpg0510</i>	(3R)-hydroxymyristoyl-[acyl carrier protein]dehydratase	-2.79
<i>lpxA1</i>	<i>lpg0511</i>	UDP-N-acetylglucosamine acyltransferase	-2.48
<i>plsB</i>	<i>lpg0551</i>	similar to 1-acyl-sn-glycerol-3-phosphate acyltransferase	-2.66
<i>lag-1</i>	<i>lpg0777</i>	O-acetyltransferase	-4.90
<i>lpg1873</i>	<i>lpg1873</i>	similar to membrane-bound lytic murein transglycosylase B precursor	-2.27
<u>MFS Transporters</u>			
<i>phfE</i>	<i>lpg0652</i>	Similar to major facilitator family transporter	-2.07
<i>lpg2501</i>	<i>lpg2501</i>	similar to multidrug resistance protein- MFS superfamily	-2.59
<u>Protein Translocation</u>			
<i>secE</i>	<i>lpg0316</i>	Preprotein translocase subunit	-2.27
<i>secF</i>	<i>lpg2000</i>	Preprotein translocase subunit	-2.17
<i>secD</i>	<i>lpg2001</i>	Preprotein translocase subunit	-2.55
<u>Heat Shock Response</u>			
<i>hslV</i>	<i>lpg0640</i>	Peptidase component of the HslUV protease	-2.96
<i>hslU</i>	<i>lpg0641</i>	ATP-dependent protease ATP-binding subunit	-2.96
<i>dnaJ</i>	<i>lpg2024</i>	Chaperone protein	-2.35
<i>dnaK</i>	<i>lpg2025</i>	Chaperone protein (HSP-7)	-2.37
<i>grpE</i>	<i>lpg2026</i>	Heat-shock protein (HSP-7 cofactor)	-3.04
<u>Other Factors</u>			
<i>nusG</i>	<i>lpg0317</i>	transcription antitermination protein	-2.11
<i>mreB</i>	<i>lpg0811</i>	Rod shape-determining protein	-3.29
<i>comL</i>	<i>lpg1186</i>	Similar to competence lipoprotein	-3.41
<i>lpg1593</i>	<i>lpg1593</i>	Similar to carbon storage regulator CsrA	-2.20
<i>nusA</i>	<i>lpg2773</i>	Transcription elongation protein	-2.18
<i>yigC</i>	<i>lpg2933</i>	oxidoreductase, 3-octaprenyl-4-hydroxybenzoate carboxy-lyase	-2.02
<i>rho</i>	<i>lpg2934</i>	transcription termination factor	-2.05
<u>Translation</u>			
<i>rplk</i>	<i>lpg0318</i>	50S ribosomal protein L11	-2.78
<i>rplA</i>	<i>lpg0319</i>	50S ribosomal protein L1	-2.43
<i>rplL</i>	<i>lpg0321</i>	50S ribosomal subunit protein L7/L12	-2.52
<i>rpsL</i>	<i>lpg0324</i>	30S ribosomal protein S12	-2.18
<i>rpsJ</i>	<i>lpg0328</i>	30S ribosomal subunit protein S1	-2.16
<i>rplC</i>	<i>lpg0329</i>	50S ribosomal subunit protein L3	-2.19
<i>rplD</i>	<i>lpg0330</i>	50S ribosomal subunit protein L4	-2.42
<i>rplW</i>	<i>lpg0331</i>	50S ribosomal subunit protein L23	-2.46
<i>rpsS</i>	<i>lpg0333</i>	30S ribosomal subunit protein S19	-2.18
<i>rpsC</i>	<i>lpg0335</i>	30S ribosomal protein S3	-1.97
<i>rplP</i>	<i>lpg0336</i>	50S ribosomal protein L16	-2.30
<i>rpmC</i>	<i>lpg0337</i>	50S ribosomal subunit protein L29	-2.23
<i>rpsQ</i>	<i>lpg0338</i>	30S ribosomal protein S17	-2.10
<i>rplN</i>	<i>lpg0339</i>	50S ribosomal protein L14	-2.67
<i>rplX</i>	<i>lpg0340</i>	50S ribosomal protein L24	-2.25
<i>rplR</i>	<i>lpg0341</i>	50S ribosomal subunit protein L18	-2.27
<i>lpg0342</i>	<i>lpg0342</i>	similar to methylated-DNA-protein-cysteine S-methyltransferase	-3.13
<i>rplS</i>	<i>lpg0343</i>	50S ribosomal protein L19	-3.02
<i>trmD</i>	<i>lpg0344</i>	Highly similar to tRNA (guanine-N1)-methyltransferase	-3.57
<i>rimM</i>	<i>lpg0397</i>	similar to 16S rRNA processing protein RimM	-3.27
<i>rpsP</i>	<i>lpg0398</i>	Highly similar to 30S ribosomal protein S16	-2.40
<i>rpL33</i>	<i>lpg0478</i>	50S ribosomal subunit protein L33	-3.91
<i>rpmB</i>	<i>lpg0479</i>	50S ribosomal protein L28	-4.44
<i>lpg0607</i>	<i>lpg0607</i>	similar to putative lysyl-tRNA synthetase	-2.27
<i>ppt</i>	<i>lpg1519</i>	putative pyrimidine phosphoribosyl transferase	-2.39
<i>rpsR</i>	<i>lpg1591</i>	30S ribosomal subunit protein S18	-2.93
<i>rpsF</i>	<i>lpg1592</i>	30S ribosomal protein S6	-3.88
<i>pyrH</i>	<i>lpg1712</i>	Uridylate kinase (UK) (Uridine monophosphate kinase)	-2.59

<i>tsf</i>	<i>lpg1713</i>	Elongation factor Ts (EF-Ts)	-2.88
<i>rpsB</i>	<i>lpg1714</i>	30S ribosomal protein S2	-3.21
<i>rpsA</i>	<i>lpg1740</i>	30S ribosomal protein S1	-1.98
<i>rpsU</i>	<i>lpg2358</i>	30S ribosomal protein S21	-2.28
<i>lpg2593</i>	<i>lpg2593</i>	similar to rRNA methylase (sun protein)	-2.26
<i>rpsT</i>	<i>lpg2636</i>	30S ribosomal subunit protein S2	-1.97
<i>rplU</i>	<i>lpg2651</i>	50S ribosomal protein L21	-5.71
<i>pth</i>	<i>lpg2653</i>	similar to peptidyl-tRNA hydrolase	-4.35
<i>rpsI</i>	<i>lpg2706</i>	30S ribosomal subunit protein S9	-2.69
<i>ppiB</i>	<i>lpg2726</i>	Peptidyl-prolyl cis-trans isomerase B	-3.18
<i>tgt</i>	<i>lpg2727</i>	Similar to queuine tRNA-ribosyltransferase	-3.25
<i>rpsO</i>	<i>lpg2769</i>	30S ribosomal protein S15	-5.29
<i>truB</i>	<i>lpg2770</i>	tRNA pseudouridine synthase B	-2.24
<i>rbfA</i>	<i>lpg2771</i>	Ribosome-binding factor A	-1.97
<i>metG</i>	<i>lpg2882</i>	methionyl-tRNA synthetase	-1.97
<i>gidA</i>	<i>lpg2889</i>	Highly similar to glucose-inhibited division protein A GidA,	-2.81
<i>thdF</i>	<i>lpg3001</i>	Similar to GTPase for tRNA modification trmE	-2.92
<i>rpmH</i>	<i>lpg3005</i>	50S ribosomal protein L34	-2.99

The data were collected from two independent biological replicates. See *Fig. S1* for qRT-PCR validation. $p < 0.01$ for all targets shown.

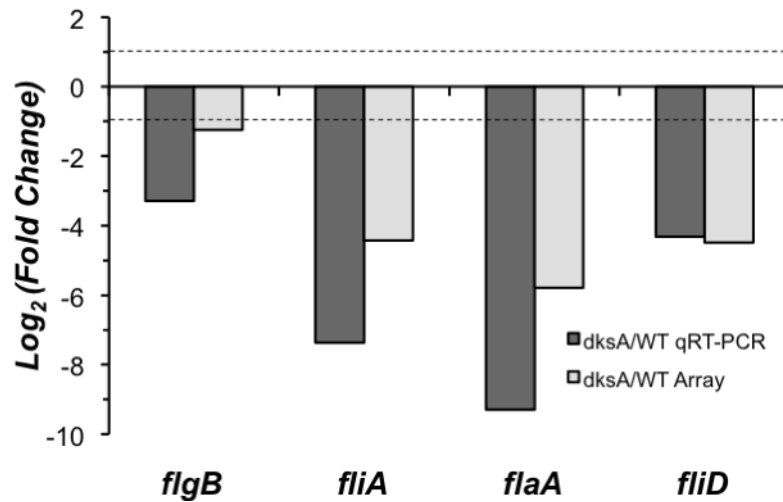
Supplemental Figure 1.



qRT-PCR validation of the *relA*^{L.p.} induction microarray data set.

L. pneumophila ppGpp⁰ mutant bacteria carrying either *pempty* or *prelA*^{L.p.} were grown in AYET to E phase (OD₆₀₀ = 1.4) then treated with 500 μM IPTG. Cells were harvested for RNA isolation at 90 min post-IPTG and qRT-PCR was performed using primers listed in *Supplemental Table 2*. The experiment was performed in duplicate and the mean relative transcript level (*prelA*^{L.p.}/*pempty*) was calculated for six targets; *letE*, *rpoS*, *flgB*, *ndk*, *lag-1* and *lpg0260*. Mean values from both the qRT-PCR and microarray experiments were log₂-transformed, reported as fold change and directly compared.

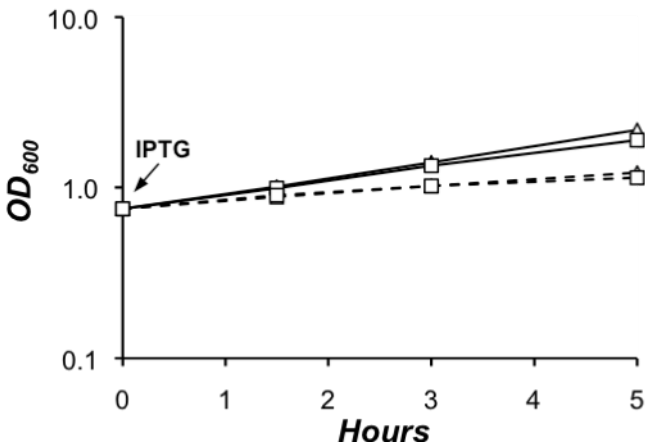
Supplemental Figure 2.



qRT-PCR validation of the PE phase *dksA*/WT microarray data set.

Cultures of WT and *dksA* mutant *L. pneumophila* were grown synchronously in AYET to PE phase until 95-100% of the WT culture was motile. Cells were harvested for RNA isolation and qRT-PCR was performed using primers listed in *Supplemental Table 2*. The experiment was performed in duplicate and the mean relative transcript level (*dksA*/WT) was calculated for four flagellar transcripts; *flgB*, *fliA*, *flaA*, and *fliD*. Mean values from both the qRT-PCR and microarray experiments were log₂-transformed, reported as fold change and plotted alongside one another. Dashed lines delineate the two-fold change cutoff.

Supplemental Figure 3



Growth of WT and *dksA* mutant *L. pneumophila* induced to express *preLA*^{E.c.}.

As an indirect measure of the amount of ppGpp produced after induction of the constitutive ppGpp synthetase, *relA*^{E.c.}, we monitored the growth response of WT and *dksA* mutant bacteria. Culturing conditions were similar to those described in *Figure 9A*. OD₆₀₀ readings of WT *empty* (solid lines, triangles), WT *preLA*^{E.c.} (dashed lines, triangles), *dksA* *empty* (solid lines, squares), and *dksA* *preLA*^{E.c.} (dashed lines, squares) were taken at 0, 1.5, 3 and 5 h post-IPTG. The slopes of the curves were calculated between 0 and 5 h post-IPTG: WT *empty* = 0.28 h⁻¹, *dksA* *empty* = 0.23 h⁻¹, WT *preLA*^{E.c.} = 0.09 h⁻¹, and *dksA* *preLA*^{E.c.} = 0.08 h⁻¹.

Supplemental Table 2.

Primers for Cloning	Sequence	Amplicon Size
dksA1 fwd.	5'-AATCTCCCCCTAAAACAATACCAC -3'	1.4 kb
dksA2 rvs.	5'-CTCGCAGCAGGGACTAAATCT -3'	
dksAi1 fwd.	5'- <u>GTCGAC</u> GAATGAAATATAGGGTCT-3'	540 bp
dksAi2 rvs.	5'- <u>AAGCTTT</u> GTTTAATGTTTCAGAGGC-3'	
dksA-pKD3a fwd.	5'-TGTATAAAACTCAGTTTCAGATACAGAA TGAAATGTGTAGGCTGGAGCTGC-3'	1.1 kb
dksA-pKD3b rvs.	5'-GGAATTTATTGTTTAATGTTTCAGAGGCA AGAAAGAACATATGAATATCCTCCTTAGTTCC-3'	
fliAP1 fwd.	5'- <u>GGATCC</u> ATGTGCAGTTAGATTACCT-3'	304 bp
fliAP2 rvs.	5'- <u>TCTAGAC</u> CAGCAGATTGTAGTTATTAGT-3'	
Real-time PCR Primers	Sequence	
letE1 fwd.	5'-AGCAGAACAATGGCTCGAAGGATG-3'	
letE2 rvs.	5'-CGGCTATCGCACCACCAATTTCAA-3'	
rpoS1 fwd.	5'-TCCTGCAGAGCTGCTAACCAATGA-3'	
rpoS2 rvs.	5'-TACCAACGTCTTCCAGTGTGCGCTT-3'	
flgB1 fwd.	5'-GCCAAGGCGTTGATTGCAAGAGAT-3'	
flgB2 rvs.	5'-TTACCGGCCATAGTTGCTGTCAGT-3'	
ndk1 fwd.	5'-TTATGGGCGCGACAAATCCCAAAG-3'	
ndk2 rvs.	5'-GCTGTCAGAACCATGCACAGCATT-3'	
lag-1a fwd.	5'-TGGTCAATAGCAGTTGAGTGGTGG-3'	
lag-1b rvs.	5'-ACCCAGAACCCAGAAACCATACCA-3'	
lpg0260a fwd.	5'-TATAACGGGCTTACAGGCTTGGCA-3'	
lpg0260b rvs.	5'-AGCAGTCAGCGCGTGAATAGAGAT-3'	
lpg2096a fwd.	5'-AGCAATTGGGAGCGAGGTGATAGA-3'	
lpg2096b rvs.	5'-AGGCTTGTGATGATCGAGCAGTG-3'	
fliA1 fwd.	5'-TCCTTAGCCTCACGCCCAAGTTTA-3'	
fliA2 rvs.	5'-ATTGGGTGCCGCGTTCTGTTTATC-3'	
flaA1 fwd.	5'-ATGACTGCACAAATTCGCGGGATG-3'	
flaA2 rvs.	5'-TGT-TTCCTGCATTGCTCCTTCAGC-3'	
fliD1 fwd.	5'-AGCGCCGACAACCTCTGTATTGACA-3'	
fliD2 rvs.	5'-TCGATGCGATAGTCACGGCAGAAA-3'	